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THE MASS CULTURE OF THE ROTIFER, *Brachionus plicatilis*, FOR USE AS FOODSTUFF IN AQUACULTURE¹

Clark T. Fontaine and Dickie B. Revera
National Marine Fisheries Service, NOAA
Southeast Fisheries Center
Galveston Laboratory
Galveston, TX 77550

ABSTRACT

Discussed is the mass culture of rotifers at the National Marine Fisheries Service, Southeast Fisheries Center, Galveston Laboratory, Galveston, Texas, in the Galveston penaeid shrimp hatchery system and for use in marine fish larval culture. Techniques are described for: 1) harvesting rotifer tanks by "skimming" the surface of the water; 2) concentrating the harvested rotifers by shocking with deionized water; and 3) storing large quantities of rotifers to be used as foodstuff by conventional freezing methods. Also discussed is a technique being developed whereby the rotifer is used as a carrier medium for various nutrients.

INTRODUCTION

Information and data on the foods of most larval crustacean and fish species, particularly at the onset of active feeding, is limited. Because newly hatched larvae (fish and crustaceans) are apparently species specific to type and size of food and are vulnerable to starvation, the food organisms used during the larval period are vitally important in determining hatchery success. A variety of organisms have been used and tested, individually and in combination, as food for larval forms. Numerous species of algae, nematodes, polychaetes, molluscan larvae, copepods, *Artemia* nauplii, and rotifers have been evaluated. In Japan (Harada 1970), most marine fish larvae are fed on a regime of oyster larvae (2-5 days post hatching), rotifers (4-25 days post hatching), and copepods (8-100 days post hatching). However, for the culture of fish larvae in the United States (Theilacker and McMaster 1971; Siefert 1972), Russia (Spectorova et al. 1974) and the United Kingdom (Jones 1972), rotifers have been found to be an excellent first food for marine fish larvae. In a variety of freshwater fish (Siefert 1972), rotifers were the dominant food of first-feeding larvae and they remained an important

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food of larger fish.

The rotifer, *Brachionus plicatilis*, has been evaluated (Sulkin 1975) as a food for larvae of the blue crab, *Callinectes sapidus*. Sulkin found that the rotifer sustained good survival through early zoeal development. However, rotifer-fed larvae did not metamorphose to the megalopa. In large production units the survival rate of zoea and mysis stages of the Kuruma shrimp, *Penaeus japonicus*, fed cultured rotifer, *B. plicatilis*, was poor (Kittaka 1975). In an experiment using small aquaria, however, cultured rotifers were found by Kittaka to be an excellent food for zoea, mysis, and early postlarvae of the Kuruma shrimp. Kittaka postulated from these studies that *B. plicatilis* was an unsatisfactory food for penaeid shrimp unless mass culture techniques could be developed to provide the number of rotifers required for mass culturing of larval penaeids.

Although methods and techniques for the culture of *Brachionus* have been documented by a number of researchers (Hirayama and Ogawa 1972; Hirayama and Kusano 1972; Hirayama et al. 1973; Hirayama and Watanabe 1973; Hirayama and Nakamura 1976; Theilacker and McMaster 1971; Howell 1973), none describe methods for the mass culture of rotifers in the quantities needed in a penaeid shrimp hatchery.

In this paper, we present our observations on the mass culture of rotifers at the National Marine Fisheries Service, Southeast Fisheries Center, Galveston Laboratory, Galveston, Texas, for use in the Galveston penaeid shrimp hatchery system (a minimum of 20×10^6 rotifers is required daily for each 2,000 liter production tank) and for use in marine fish larval culture. Additionally, techniques are described for harvesting, concentrating and storing these large quantities of rotifers to be used as foodstuff. A brief discussion is also presented on a technique being developed whereby the rotifer is used as a carrier medium for various nutrients.

MATERIALS AND METHODS

Ten fiberglass tanks (1.5 x 0.6 x 0.6 m), placed in an open shed without temperature control, were used to culture rotifers. The tanks were filled to a depth of 0.5 m with unfiltered seawater (472.6 liters) and were stocked with rotifers at a density of one rotifer/ml. Initially, 50 g of torulose yeast was added to each tank daily (0.04 g/liter). The initial feeding and the daily feeding rates were arbitrarily derived. The total weight of dry yeast to be fed in the 10 tanks was first suspended in 3,000 ml of water and mixed thoroughly; 300 ml of this mixture was then added to each tank and the culture water stirred with a wooden paddle. No *Chlorella* or other algae were fed to any of the rotifer cultures; however, after a few days, algae were growing in all tanks. Immediately after stirring, water samples were taken to measure the population density in each tank. Approximately 2 hours after stirring, the rotifers would come to the surface of the culture water and could be skimmed off. A second population estimate from the surface skim was made at that time and the tanks were harvested when the surface counts were more than 4,000 rotifers/ml. The harvest, or concentrate, from all tanks was combined and strained through a fine mesh net (0.0947 mm mesh size) to remove insect larvae and debris. All rotifers processed in this study were placed in 200 ml plastic cups at 20×10^6

per cup and frozen at 4°C until needed. A concentration of 20×10^6 rotifers is needed to provide the desired quantity of rotifers (10/ml) for a 2,000-liter penaeid shrimp hatchery production tank (C. R. Mock, personal communication, 1978).

For harvests yielding less than 0.6 liter of concentrate, a count was first made to determine the number of rotifers/ml. A volume of the harvest calculated to contain 20×10^6 rotifers was then added to a 2-liter separatory funnel and an equal volume (1:1 ratio) of deionized water was added and thoroughly mixed. Within 15 min the rotifers in the separatory funnel had precipitated from the water column and could be removed from the bottom (Fig. 1).

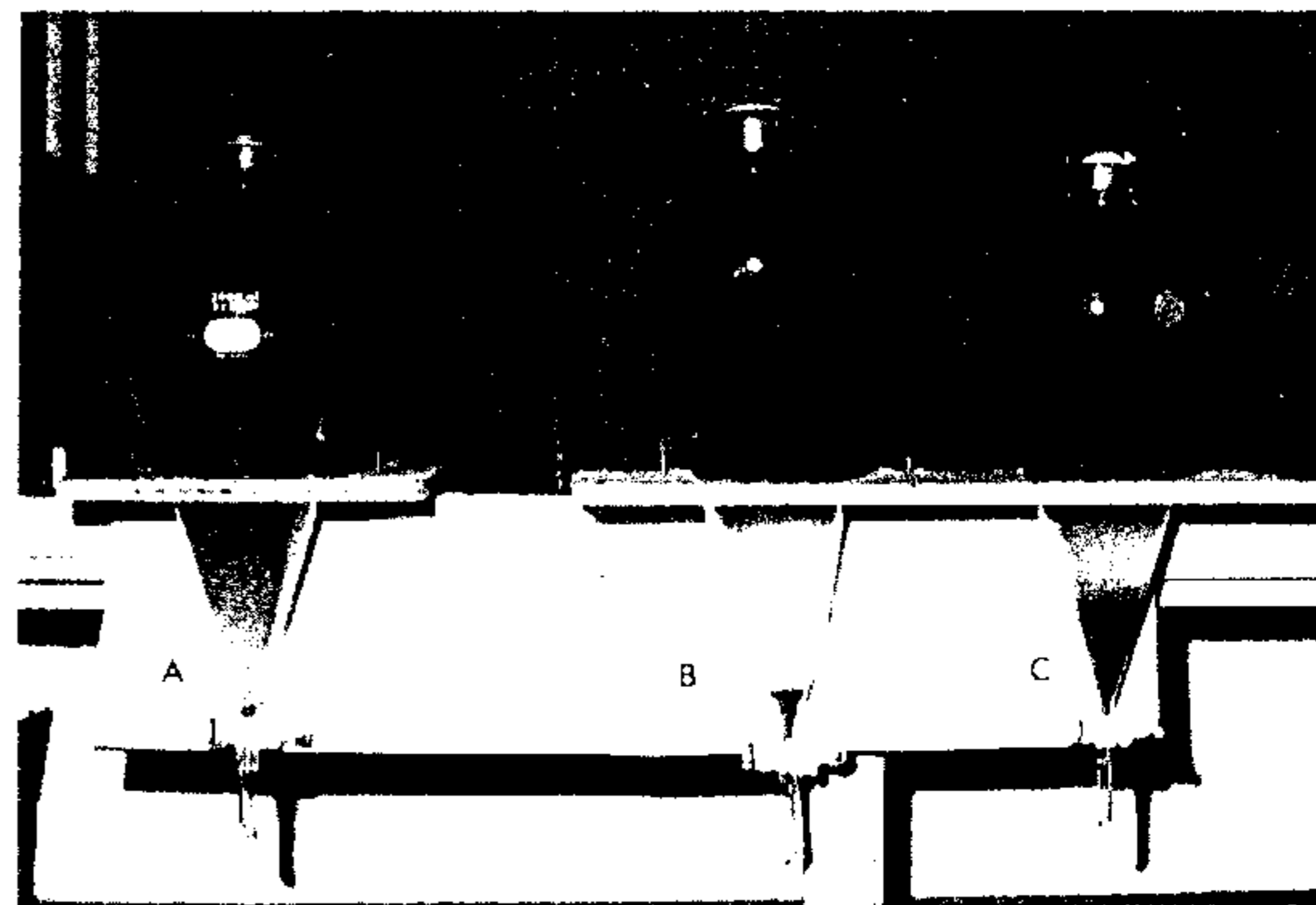


Figure 1. Concentration of rotifers using deionized water in separatory funnels: (A) one min, (B) 5 min, (C) 15 min.

Harvests larger than 0.6 liter were processed similarly, using inverted 19-liter glass carboys with the bottom removed (Fig. 2). In this instance, counts to determine rotifers/ml were done after concentration had occurred. Not all of the rotifers would fall to the bottom in the large carboys. The water remaining after drawing off the precipitated rotifers was stirred and allowed to stand for an additional hour. A pure egg-bearing population of rotifers could then be skimmed from the surface and returned to the culture tanks.



Figure 2. Glass carboy (18.9 liter) used to concentrate large amounts of rotifer harvest.

RESULTS

The estimated total population growth (rotifers/ml/time) in each of the 10 tanks and measurements of rotifer density taken from the surface 2 hours after the tanks were stirred are shown in Table 1. According to the estimated population, 50% of the rotifer population had risen to within 25 mm of the surface in each culture tank within 2 hours of stirring (Table 2). The estimated number of rotifers taken from the surface, however, shows that at each harvest only about 10% of the total population was removed. Using the techniques described here, all 10 tanks could be harvested every 24 hours with a total harvest of between 10×10^7 and 24×10^7 rotifers.

Table 1. Population Growth of Cultured Rotifers (per ml) over a 22-Day Period. Upper data is number of rotifers/ml for entire tank; lower figures are number of rotifers/ml in surface 2 hours after stirring.

Date	Day	Tanks									
		1	2	3	4	5	6	7	8	9	10
No. of rotifers/ml for entire tank											
7/21/77	0	1	1	1	1	1	1	1	1	1	1
7/25/77	4	8	4	6	6	1	2	1	2	1	1
7/27/77	6	19	25	12	24	2	1	1	2	1	39
7/30/77	9	38	40	27	32	12	12	12	9	5	49
8/01/77	11	64	72	62	68	50	60	48	60	33	220
8/04/77	14	272	303	291	301	268	249	306	405	198	507
8/12/77	22	312	321	316	359	293	305	314	481	263	583
No. of rotifers/ml in surface 2 hours after stirring tank											
8/04/77	14	2768	3941	1750	4562	3321	1982	4815	5405	1574	9643
8/12/77	22	7960	8941	8540	9231	7159	8104	8407	9862	7930	10169

Table 2. Comparison of Total Population, Surface Population, and the Numbers of Rotifers Harvested from Each Tank on the 22nd Day after Inoculation (number $\times 10^7$)

	Tanks									
	1	2	3	4	5	6	7	8	9	10
Total tank	15.0	15.4	15.2	17.3	14.1	14.7	15.1	23.1	12.6	28.0
Surface	8.0	8.9	8.5	9.2	7.2	8.1	8.5	9.9	7.9	10.2
% in Surface	53	58	56	53	51	55	56	43	63	36
Harvested	1.6	1.7	1.7	1.8	1.4	1.6	1.7	1.9	1.6	2.0
% Harvested	11	11	11	10	10	11	11	8	13	7

The tanks set up in the manner described here did not contain monocultures of the rotifer, *Brachionus*. There were several other genera of rotifers present in the culture, the most abundant being *Brachionus plicatilis* and *Lepadella ovalis* (Randy Phillips, personal communication, 1978, U.S. Dept. Interior, Fish and Wildlife Service, San Marcos, Texas). Additionally, there was a vast array of ciliates, flagellates, nematodes, and insect larvae present in the culture tanks. Most of the nematodes and all the insect larvae were removed when the harvest was passed through the harvest net (mesh size 0.0947 mm). The dilution of the rotifer harvest with deionized water did not appear to affect the ciliates, and they remained in the water column while the rotifers settled to the bottom where they were easily collected.

The initial temperature of the culture water was 28°C and the salinity 30 ppt. During the study the temperature increased to 30°C and the salinity to 44 ppt. This variation in temperature and salinity did not appear to affect the population increase of rotifers. There was a

considerable buildup of hydrogen sulfide in all culture tanks that necessitated washing the concentrated rotifers before introducing them into the hatchery tanks. The washing was done by placing the concentrate in a fine mesh plankton bag (mesh size 0.0685 mm) and dipping it several times in a bucket of fresh seawater. Only a small number of rotifers would pass through the net during the washing process. Over a period of 4 months (May-August) approximately 9×10^5 rotifers were harvested from our tanks. Many of the rotifers were fed alive; however, 1.7×10^9 were frozen for future use.

The greatest density of rotifers in our tanks occurred during the summer months (28-30°C). In the fall when ambient temperatures fell to 25°C the numbers of rotifers began to decline. There were no active rotifers in the culture tanks when the average water temperature reached 20°C. However, there was a large number of dormant eggs present in the bottom sediments in each tank. It was decided, therefore, not to agitate or disturb the tanks during the winter months so that we could investigate rotifer over-wintering. The following spring when the water temperature reached 20°C the dormant eggs hatched. When the water temperature again attained 25°C, rotifer blooms were noted in all tanks. With the commencement of supplemental feeding of torulose yeast, dense populations of rotifers were again obtained.

DISCUSSION

The techniques described here for concentrating and storing rotifers have worked well at the Galveston Laboratory. Frozen rotifers have been held for as long as one year and then used successfully in shrimp rearing experiments (Mock, personal communication). Rotifers that have been concentrated using deionized water can be revitalized in seawater if they are removed from the deionized water solution within 30 min. Additionally, a more efficient method of harvesting from the surface might be desirable. For instance, Howell (1973) found that the population density of *B. plicatilis* doubled approximately every 1-5 days; i.e., 33% of the population was produced over the preceding 24 hours, thus, assuming the continuation of that rate of reproduction, a harvesting rate of 33% of the culture per day could be adopted. Furthermore, in 10 liters of culture Howell harvested a mean daily yield of 370,000 rotifers or an average of 37 rotifers per ml.

Frozen rotifers, fresh concentrated rotifers, and live rotifers taken directly from culture tanks at our station have been fed to: *Macrobrachium* larvae in hatchery tanks (Mock et al., manuscript); larvae and postlarvae of the white shrimp, *Penaeus setiferus*, in hatchery tanks and raceways; redfish fry, *Sciaenops ocellata*, in hatchery tanks; and striped bass fry, *Morone saxatilis*, in raceways (Mock, intra-laboratory reports). All larval and post-larval forms of crustaceans appeared to feed as well on frozen as on live rotifers. Finfish larvae, however, did not appear to accept the frozen rotifers as foodstuff while they would actively feed upon live rotifers.

Sulkin (1975) and Sulkin and Epifanio (1975) have evaluated various diets, including rotifers, for rearing larvae of the blue crab, *Callinectes sapidus*. The rotifer, *Brachionus plicatilis* Muller, sustained good survival through early zoeal development; however, rotifer-fed larvae did not metamorphose to the megalopa. Sulkin showed that *Artemia salina*

nauplii contain 2-3 times as much lipid per dry weight as do rotifers. A metabolic requirement for lipid of brachyuran larvae late in development may be indicated. Additionally, Kittaka (1975), in his work on the Kuruma shrimp in Japan, stated that crustaceans are incapable of biosynthesizing cholesterol and that they require sterol as a diet. Cholesterol is a precursor of a molting hormone in crustaceans that are capable of converting photosterol to cholesterol.

We are, therefore, presently evaluating the rotifer, *Brachionus*, as a carrier medium for various foodstuffs in order to improve their nutritional value. The process involves purging the rotifers in clean seawater for 24-48 hours, resuspending the rotifers in a dense mixture of nutrients, harvesting, and freezing. The cleansed, starving rotifers literally pack themselves with the suspended nutritive material and are then harvested and frozen before digestion by the rotifer can occur. We have successfully packed rotifers with algae, *Tetraselmis chui* and *Chlorella* sp., and with torulose yeast. Rotifers packed with *Tetraselmis* and frozen have been fed to striped bass larvae, but the technique has not been evaluated. Tentatively, we have termed the process "micro encapsulation."

The rotifer, *B. plicatilis*, has been demonstrated by a number of researchers to be an excellent first food for several genera of marine and freshwater fish larvae. Jones (1972) found that *Artemia* nauplii were unsuitable for first-feeding turbot (*Scophthalmus maximus* L.) and brill (*S. rhombus* L.) larvae because of their size; the larvae preferred particles less than 0.2 mm in length. Rotifers were used almost exclusively as a first food for larvae. When the larvae had grown for a time on rotifers they were then transferred to a diet of brine shrimp nauplii. Although Sulkin (1975) found rotifers to be an unsuitable diet for blue crab larvae and Kittaka (1975) had similar results feeding rotifers to Kuruma shrimp larvae, we believe that the rotifer will make an excellent intermediate food for penaeid shrimp larvae. The value of rotifers as an intermediate food between algae and *Artemia* nauplii for penaeid larvae will depend, in part, upon the success of encapsulating within rotifers the nutritional requirements for shrimp.

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COMPARATIVE GROWTH AND SURVIVAL OF BROWN SHRIMP CULTURED WITH FLORIDA POMPANO, BLACK DRUM AND STRIPED MULLET

Karen S. Rossberg and R. Kirk Strawn
Department of Wildlife and Fisheries Sciences
Texas A&M University
College Station, TX 77843

ABSTRACT

Brown shrimp (*Penaeus aztecus*) were grown at densities of 3000/ha in polyculture with fish in flow-through 0.1 ha ponds. Shrimp, 104.8 ± 6.81 mm in total length, were stocked with Florida pompano (*Trachinotus carolinus*), 67.6 ± 9.32 mm in standard length (SL); Florida pompano, 58.0 ± 6.13 mm SL, and black drum (*Pogonias cromis*), 91.9 ± 11.17 mm SL; black drum, 86.3 ± 10.65 mm SL; and black drum, 93.7 ± 7.92 mm SL, and striped mullet (*Mugil cephalus*), 74.5 ± 26.36 mm SL. Densities were 5000/ha for Florida pompano and black drum, and 500/ha for striped mullet. Shrimp survival ranged from 19.0 to 63.3%. Average survivals, all treatments, were poorest with Florida pompano and best with black drum in fish monocultures. Growth was excellent, averaging 0.17 g/day and similar with all fish species and combinations.

INTRODUCTION

Shrimp and fish polyculture is practiced commercially in Taiwan and the Philippines (Liao and Huang 1970; Anonymous 1975). Gundermann and Popper (1977) studied the feasibility of rearing three species of penaeid prawns with three fish species. All prawns were maintained in monoculture ponds containing fish. All prawn species grew and gained substantial weight in the presence of fish. The success of such operations depends upon the choice of the most suitable species as well as the relative stocking sizes of the shrimp and fishes. Even carnivorous fishes such as Florida pompano (*Trachinotus carolinus*) and black drum (*Pogonias cromis*) may be successful counterparts in polyculture with shrimp under certain conditions. For example, Tatum and Trimble (1977) found the most productive stocking method was to rear post-larval brown shrimp (*Penaeus aztecus*) separately before stocking them with the juvenile Florida pompano in grow-out ponds. They concluded that if only the fish are fed, food conversion efficiencies (FCE) might be increased.